AWARD NUMBER: W81XWH-16-1-0702

TITLE: Optimization of Delayed Tolerance Induction in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation

PRINCIPAL INVESTIGATOR: Curtis L Cetrulo, MD, FACS

RECIPIENT: Massachusetts General Hospital

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Fort Detrick, Maryland 21702-5012

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Optimization of Delayed Tolerance Induction in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation

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W81XWH-16-1-0702

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5d. PROJECT NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)

The Massachusetts General
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8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Materiel Command
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12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This research project addresses the FY15 RTR Focus Areas of Understanding mechanisms of immune rejection and Immunomodulation approaches and mechanisms (e.g., tolerance induction, chimerism).
Tolerance of kidney allografts has been achieved in nonhuman primates (NHPs) using a the delayed period protocol, i.e combination of post-transplant non-myeloablative conditioning and donor bone marrow transplantation four months later (DBMT).

15. SUBJECT TERMS
None listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Vascularized composite allotransplantation (VCA) has emerged as a viable option for restoring form and function in patients with devastating soft tissue defects. To date, 40 faces and 112 hand/upper extremity transplants have been performed worldwide, with promising short- to intermediate-term functional and immunological outcomes. Nevertheless, the requirement for long-term immunosuppressive therapy to maintain the allograft increases the risk of related side effects such as infections, metabolic complications or even malignancies. Consequently, it is essential to develop a strategy to achieve immune tolerance, to obviate the requirement for long-term maintenance immunosuppression. T cell co-stimulation blockade (CoB) arose as an attractive concept to induce transplant tolerance in the 1990s and has been developed and used successfully in murine heart and islet cell transplant models. The administration of donor bone marrow (BM) cells in combination with CoB appears to be the most promising approach to achieve a state of tolerance through mixed chimerism, as indicated by various small and large animal solid organ transplant models. We propose to develop clinically relevant strategies using CoB (belatacept) and donor bone marrow cells to induce mixed chimerism in an established swine model of VCA.

We hypothesize that the attainment of transplantation tolerance, defined as the absence of destructive immune responses against a transplanted organ or tissue without the requirement for immunosuppression, would not only allow successful withdrawal of immune medications but also potentially negate the development of chronic rejection.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Vascularized composite allotransplantation, mixed chimerism, co-stimulatory blockade, bone marrow transplant, immunologic tolerance, fasciocutaneous flap

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Our objectives are: (1) to add co-stimulatory blockade to promote both successful engraftment after donor bone marrow cell infusion to achieve mixed chimerism and thus tolerance of VCA and (2) to apply the day 0 protocol across the range of MHC barriers that may be encountered clinically to demonstrate the robustness of this approach.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We are awaiting the pending technical amendment to be approved before moving forward with the project. We expect to complete the project on schedule.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

IACUC and ACURO approval

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*

- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

We amended the SOW to replace the delayed tolerant protocol to day 0 protocol. The reasons of these changes are our promising results with the day 0 protocol from our laboratory. We managed to induce long-term survival immunosuppression-free with development of mixed chimerism in two swine (class 1 mismatch).

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name	Project Role	Person month worked	Contribution to the project
Curtis Cetrulo	PI	0.24	Overall design and direction of proposed studies, interpretation of results.
Josef Kurtz	Co-Investigator	3.0	Assessment of transplant recipients, supervision of work performed by research fellow, assists with interpretation of results.
Alexandre Lellouch	Research Fellow	0.16	Assist in surgical procedures, analyses of immune responses, interpretation of results.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Current Support Changes for the PI, Co-I or Other Senior/Key Personnel Changes in Current Support	
Curtis Cetrulo	Change: Extended Shriners Hospital for Children, Boston grant 85230-BOS-14 "Immunology of Hand and Face Transplantation for Burns" Role: PI Effort: 0% Date: 01/01/14-12/31/17 No impact
Curtis Cetrulo	Change: Received DoD grant W81XWH-17-1-0680 "Development of a Supercooled Limb Preservation Protocol" Role: Co-PI Effort: 2% Date: 09/01/17-08/31/20 No impact
Curtis Cetrulo	Change: Received DoD grant W81XWH-17-1-0454 "GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps" Role: PI Effort: 1% Date: 09/15/17-09/14/19 No impact
Curtis Cetrulo	Change: Received MTF Established Investigator Grant "Costimulation Blockade-Based Regimens of Mixed Chimerism to Overcome Split Tolerance in VCA" Role: PI Effort: 5% Date: 08/01/17-07/31/20 No impact
Curtis Cetrulo	Change: Received XenoTherapeutics, Inc. Sponsored Research Agreement Role: PI Effort: N/A Date: 12/15/16-12/14/19 No impact
Curtis Cetrulo	Change: Received Shire HGT, Inc. Sponsored Research Agreement

	Role: PI Effort: N/A Date: 11/01/16-10/31/19 No impact
Curtis Cetrulo	Change: Received DoD grant W81XWH-16-1-0702 "Optimization of Delayed Tolerance Induction in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation" Role: PI Effort: 2% Date: 09/15/16-09/14/18 No impact
Curtis Cetrulo	Change: Received AxoGen, Inc. Clinical Trial Agreement (switched to PI) "A Multicenter, Prospective, Randomized, Subject and Evaluator Blinded Comparative Study of Nerve Cuffs and Avance® Nerve Graft Evaluating Recovery Outcomes for the Repair of Nerve Discontinuities (RECON)" Role: PI Effort: N/A Date: 11/25/15-11/24/18 No impact
Curtis Cetrulo	Change: Ended DoD grant W81XWH-13-2-0062 "Tolerance in Nonhuman Primates by Delayed Mixed Chimerism" Role: PI Effort: 1% Date: 09/15/13-09/14/17 No impact
Curtis Cetrulo	Change: Ended DoD grant W81XWH-13-2-0060 "Immunomodulation Tolerance Induction after VCA Using Biologic Agents (CTLA4-IG) and Donor BM Cells" Role: PI- subcontract Effort: 1% Date: 09/15/13-09/14/17 No impact
Curtis Cetrulo	Change: Ended DoD grant W81XWH-12-2-0037-P00003 "A Novel Protocol for Upper Extremity Restoration by Transplantation with Intent for Tolerance Induction" Role: PI- subcontract Effort: 1% Date: 09/30/12-09/29/17 No impact
Curtis Cetrulo	Change: Ended DoD grant W81XWH-13-2-0053 "Towards a Preclinical Large Animal Tolerance Protocol for Vascularized Composite Allotransplantation in Swine" Role: PI- subcontract Effort: 1.5% Date: 09/18/13-09/17/17 No impact

Josef Kurtz	Change: Ended DoD grant W81XWH-12-2-0037-P00003 "A Novel Protocol for Upper Extremity Restoration by Transplantation with Intent for Tolerance Induction" Role: Investigator Effort: 25% Date: 09/30/12-09/29/17 No impact
Josef Kurtz	Change: Ended DoD grant W81XWH-13-2-0062 "Tolerance in Nonhuman Primates by Delayed Mixed Chimerism" Role: Investigator Effort: 50% Date: 09/15/13-09/14/17 No impact
Josef Kurtz	Change: New DoD grant W81XWH-15-1-0281 "Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Non-Human Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol" Role: Investigator Effort: 25% Date: 09/15/15-09/14/18 No impact
Josef Kurtz	Change: Received DoD grant W81XWH-17-1-0454 "GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps" Role: Investigator Effort: 25% Date: 09/15/17-09/14/19 No impact
Josef Kurtz	Change: Received Shire HGT, Inc. Sponsored Research Agreement Role: Investigator Effort: 25% Date: 11/01/16-10/31/19 No impact

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*

- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None

Optimization of Tolerance protocol in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation

Log Number: RT150065

Award Number: W81XWH-16-1-0702

PI: Curtis L. Cetrulo, Jr., M.D., FACS **Org**: Massachusetts General Hospital **Award Amount:** $449,995

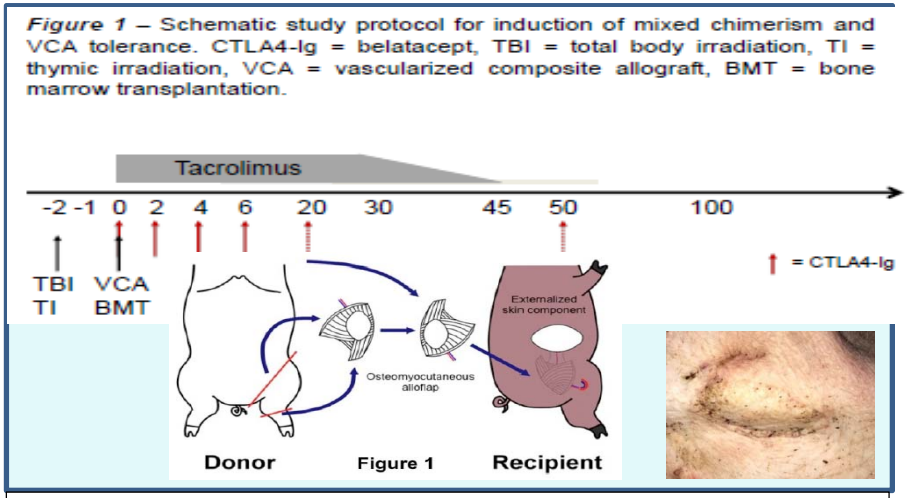
Study/Product Aim(s)

- To achieve proof-of-concept of the day 0 induction protocol in generating durable mixed chimerism for immunosuppression-free VCA tolerance and survival in swine
- To investigate the role of difference MHC class mismatches in VCA

Approach

We will modify our previously successful tolerance induction protocol using CM-PBMC into a clinically-relevant, mixed chimerism approach by bone marrow transplantation across various MHC barriers to mirror the clinical challenges of MHC matching for donor-recipient pairs in VCA.

Figure 1 – Schematic study protocol for induction of mixed chimerism and VCA tolerance. CTLA4-Ig = belatacept, TBI = total body irradiation, TI = thymic irradiation, VCA = vascularized composite allograft, BMT = bone marrow transplantation.



Accomplishment: Obtained IACUC approval

Timeline and Cost

Activities / CY	16	17	18	19
Perform VCA in class I mismatch recipients				
Perform VCA in full MHC mismatch				
Investigate chimerism, VCA survival, complications, *in vitro* immune status				
Complete analysis, prepare manuscript for submission				
Estimated Budget ($K)	**$8**	**$272**	**$170**	**$0**

Updated: October 15th, 2017

Goals/Milestones

CY16 Goals

☐ Obtain IACUC and ACURO approval

☐ Establish induction protocol for immunosuppression-free VCA tolerance in full mismatch swine

CY17 Goal

☐ Validate induction protocol across various MHC mismatches

CY18 Goal

☐ Identify possible mechanisms behind VCA acceptance or rejection in the context of different MHC mismatches

Comments/Challenges/Issues/Concerns

- N/A

Budget Expenditure to Date

Projected Expenditure: $55,662

Actual Expenditure: $55,662